Identification and Determination of the Major Acids of the White Potato

The major potato acids were identified and a method for their determination was developed for use in studying changes in potato composition during processing and during preprocessing storage. Glutamic, pyroglutamic (possibly an artifact), malic, citric, and oxalic acids were isolated and characterized; aspartic, chlorogenic, and phosphoric acids were identified by chromatographic or other means. An ion exchange method for determining these acids (except for chlorogenic acid) and a small-scale, qualitative, paper chromatographic method for following eluates from columns were developed.

PROCESSED PRODUCTS now comprise about 30% of the potatoes consumed as food in the United States, as compared to about 2% in 1940. This increase in processing has led to problems in connection with the browning of chips and French fried potatoes, aftercooking discoloration, and the production of off-flavors. Recent work (*1, 3, 11, 13*) has indicated that nonnitrogenous organic acids, in addition to amino acids as previously reported, may also play a part in browning reactions and in aftercooking discoloration.

Published qualitative analyses for potato acids have been based mainly on chromatographic methods or, especially in the older literature, on chemical reactions of varying degrees of specificity; few actual characterizations were undertaken. Several authors (*10, 14*) identified chlorogenic acid by ultraviolet spectrophotometry of material separated on papergrams; Kuc *et al.* (*15*) and Craft *et al.* (*8*) identified both chlorogenic and caffeic acids by this means. Curl and Nelson (*9*) isolated citric, isocitric, and malic acids by distillation of their ethyl esters and characterized them as hydrazides. Oxalic acid was isolated and identified by crystallographic methods after precipitation as calcium oxalate. The above works are only those involving at least partial characterizations; glutamic, aspartic, phosphoric, and other acids reported to occur in potatoes are mentioned in more general reviews such as those of Burton (*6*) and of Schwimmer and Burr (*22*).

Of the published quantitative methods for the determination of organic acids in plant materials, ion exchange chromatography has several advantages over silica gel methods (*19*). However, the ion exchange methods could not be used without modification, since they did not include all the major potato acids, and since some of the procedures used were too time-consuming for a routine method.

The purpose of the present work was to isolate and characterize the major potato acids and to develop a method for their determination in studies concerning changes occurring during processing and during preprocessing storage. A quantitative ion exchange method is presented, and also a small-scale, qualitative, paper-chromatographic method for following eluates from the ion exchange columns.

Identification of Acids

Twenty-five pounds of Irish Cobbler potatoes, washed and freed of sprouts and bad spots, were ground in a Fitzmill comminuting machine and heated, with stirring, for $^1/_2$ hour near the boiling point with enough alcohol to produce a 70% solution. After filtration the residue was extracted in the same way with 16 liters of 70% alcohol. The combined extracts were concentrated in a circulating evaporator to remove the alcohol.

An acid fraction was obtained from the extract by the use of ion exchange resins according to the method of Porter *et al.* (*20*), except that columns containing 1600 ml. of resin were used and the acids were eluted from the anion exchange resin with 0.1*N* NaOH. The eluted acids were then fractionated on silica gel by a modification of the technique of Marvel and Rands (*17*). Silicic acid (Mallinkrodt, 100 mesh) was treated to remove fines according to the method of Bulen *et al.* (*5*). Column size and amounts of silica gel and eluting solvents were scaled up tenfold over the specifications of Marvel and Rands. An amount of the acid fraction (sirupy) equivalent to 2 grams of acids was mixed with 20 grams of silica gel and an appropriate amount of water and added to the top of the column. The solvent schedule was similar to that used by Marvel and Rands, but additional, more polar solvents were needed to elute all the acids. Aliquots of the eluate fractions were titrated. The elution schedule and the identity of the peaks, as determined by paper chromatography, are shown in Table I. Data for each peak adjoin the data for the particular solvent with which it was eluted. The fractionation was repeated with an additional 2 grams of acids on a second silica

Table I. Fractionation of Potato Acids on Silica Gel

Eluting Solvent	Liters	No.	Elution vol., liters	Identification
Chloroform–butyl alcohol				
80:20	1			
75:25	1			
70:30	1	1	2.3	Pyroglutamic[a]
60:40	1			
50:50	1	2	4.5	Malic
30:70	1	3	5.5	Citric
		4	7.1	Phosphoric
Butyl alcohol–H_2O, 100:15	2.5	5	8.2	Oxalic[a]
Butyl alcohol–methanol–H_2O				
95:5:20	1	6	9.3	Glutamic[a]
90:10:30	0.2			
70:30:60	1	7	10.2	Unidentified[a]

[a] Impure mixtures.

gel column, and corresponding peaks of the two fractionations were combined.

In the following acid identifications, melting points were determined with a Kofler micro hot stage; comparisons of x-ray diffraction powder patterns were made between knowns and unknowns crystallized in the same manner.

Glutamic Acid. A small quantity of platelets crystallized from very dilute solution (peak 6 before concentrating). These were filtered out and washed with acetone. The infrared absorption curve (Nujol mull) of the unknown was almost identical to that of authentic L-glutamic acid (slight differences possibly due to the solid state), but considerably different from that of L-aspartic and DL-aspartic acids. The x-ray diffraction pattern was also identical to that of L-glutamic acid.

Pyroglutamic Acid. Since peak 1 was relatively impure, it was rechromatographed on silica gel. Titration of the eluate showed pyroglutamic acid to be the major constituent. Fractions containing pyroglutamic acid were combined, passed through Dowex 50 (H^+ form) to remove sodium ions, and adsorbed on a small Dowex 2 column in the OH^- form. The material was then freed from indicator (phenolsulfonephthalein) by selective elution with 1*N* acetic acid and crystallized from a methyl ethyl ketone–carbon tetrachloride mixture. M.p. 161.5° to 163.5° C. [Lit. 160° C. (*25*)]. The x-ray diffraction pattern was identical to that of authentic L-pyroglutamic acid. This acid does not necessarily occur in the potato, but may be an artifact derived from glutamine or glutamic acid.

Malic Acid. The residue from peak 2 was converted to an anilide by the method of Macallum (*16*). The material crystallized from the reaction mixture after adding alcohol and seeding with malanilide. M.p. 202° to 203° C. Authentic malanilide melted at 202.5° to 204° C. X-ray diffraction patterns of malanilide made from the potato acid and of authentic malanilide were identical.

Citric Acid. Peak 3 crystallized upon evaporation. The crystalline mass was dissolved in hot alcohol, decolorized with Norit, and crystallized twice from acetone-chloroform (ca. 8:3). M.p. 152.3° to 154.0° C. [Lit. 153° C. (*18*), 156° to 157° C. (*4*)]. The potato acid had the same x-ray diffraction pattern as authentic citric acid.

Phosphoric Acid. Peak 4 gave the same R_f as orthophosphoric acid on chromatograms and the same color reactions with ammonium molybdate–spray (*2*) and ammoniacal $AgNO_3$–spray reagents. It also gave a yellow precipitate when treated in aqueous solution with acidic ammonium molybdate.

Oxalic Acid. This acid, as indicated by R_f's on chromatograms, probably occurred in mixture in peak 5. It was not isolated from this peak, but from a fraction obtained by an ion exchange method. A 70% acetone extract of peeled Katahdin potatoes was concentrated to remove acetone and adsorbed on a Dowex 1 column in the formate form. The oxalic acid appeared, among other acids, in a fraction eluted by 1*N* HCl but not by 6*N* HCOOH. It was purified chromatographically on large sheets of acid-washed Whatman No. 1 filter paper. Zones containing the oxalic acid were cut out and eluted. The acid was then decolorized with Norit and precipitated with calcium acetate. The precipitate gave an x-ray diffraction pattern similar to that obtained from calcium oxalate prepared in the same manner.

Chlorogenic Acid. This acid was identified by its R_f on chromatograms, its strong fluorescence, and its relatively weak acid reaction with bromophenol blue. It appeared in extracts of potatoes (both peeled and unpeeled) fractionated on Dowex 1 (formate form), but did not give a peak in the analytical method described below. It also did not appear in the silica gel chromatography, apparently having been destroyed by the alkaline conditions used in obtaining the acid fraction.

Aspartic Acid. Aspartic acid was identified in ion exchange fractionations by its R_f on chromatograms, elution volume, and color reaction with ninhydrin.

Paper Chromatography

The method here described has the advantages of being relatively rapid and of not giving streaks with relatively strong acids such as oxalic and phosphoric.

The apparatus consists of a 4-inch diameter 6-inch high glass cylinder containing an 80 × 40 mm. crystallizing dish and a 4 × 11 inch wick of Whatman No. 1 filter paper placed at the bottom of the cylinder around its inner circumference. The acids are spotted on a $5^3/_8$-inch square of S. & S. 589 Blue Ribbon filter paper along a line $^1/_2$ inch from the bottom. Somewhat better results are obtained if the starting line is placed so that elution is parallel rather than perpendicular to the screen lines. After the sheet is stapled into a cylinder (with the edges not touching) and left standing in the crystallizing dish, the apparatus is placed on a cork ring and 5 ml. of the aqueous phase of butyl alcohol–90% formic acid–water (10 : 2 : 5) is allowed to run down over the wick. The cylinder is covered with a gasket cut from neoprene sheeting, a piece of plate glass, and a weight cut from lead pipe. A stoppered hole in the glass cover allows for later addition of the eluting solvent.

After 1 hour of equilibration, 5 ml. of the organic phase of the solvent is introduced into the crystallizing dish by a funnel with a drawn-out stem. After development, which takes about 3 hours, the sheet is opened, dried 2 to $2^1/_2$ hours in a hood, and sprayed with 0.04% bromophenol blue adjusted to slight alkalinity. The solvent front may be marked under ultraviolet light any time after drying.

Ion Exchange Chromatography

Procedure. The ion exchange procedure is based on that of Busch *et al.* (*7*), Palmer (*19*), and Hulme and Wooltorton (*12*). The elution schedule was modified to adapt it to the acids in the potato, and the apparatus was modified for greater convenience.

Fines are removed from Dowex 1 (X8, — 400 mesh) by stirring with water and decanting until almost all the remaining material settles out in half an hour. The resin is eluted with 1*N* sodium acetate until free of chloride ions and then washed until free of sodium ions. The treated resin is stored under water. Immediately before actual use, enough resin for a column (ca. 10 ml.) is stirred with 6*N* acetic acid and let stand 15 minutes. The supernatant acid is decanted and the resin is stirred with water and poured in three portions into a 7.8-mm. I.D. glass column. Each portion is allowed to settle for 10 minutes and then compacted by downflow before adding the next. Total resin height is 13.5 cm. The column is washed with water until the eluate is neutral and then with 50 ml. more. A flow rate of 1 ml. per minute is used for both washing the column and adding potato extract to it.

Potatoes are given five batchwise extractions with 70% alcohol and two hot extractions in a Soxhlet apparatus, according to the method of Talley *et al.* (*24*). An amount of extract equivalent to 10 grams, fresh weight, of peeled potatoes is condensed to remove

alcohol, shaken 15 minutes with 0.04 gram of Santocel C, filtered through Whatman No. 12 folded filter paper, and added to the resin column. The column is then washed with 50 ml. of water.

A 100-ml. volumetric flask (holding ca. 105 ml.) is used as a mixing chamber for the gradient elution apparatus. A magnetic stirring bar is placed in the bottom of the water-filled flask, and a rubber stopper, through which is passed 4-mm. O.D. inlet and outlet tubes, is placed on the top. The inlet tube leads from the leveling-bulb reservoir to just above the stirring bar. The outlet tube connects to a length of $^1/_{16}$-inch bore, $^1/_{32}$-inch wall rubber tubing which passes through a peristaltic action pump driven by a 3-r.p.m. synchronous motor and thence is connected to the top of the resin column. The pump has 12 rollers, each 3.5 cm. from the central axle. The surface of the rubber tubing is lubricated with silicone grease. With the use of the pump the flow rate (approximately 0.8 ml. per minute) remains constant during the course of a run, whereas gravity or air-pressure gives a varying flow rate.

Water is placed in the reservoir and allowed to flow through the apparatus in order to remove air. When the water level has dropped below the reservoir, 40 ml. of 2*N* acetic acid are added. To prevent premature mixing, a bubble is left between the water and the acetic acid, and between the succeeding eluting solvents—namely, 70 ml. of 6*N* formic acid and 100 ml. of 0.5*N* hydrochloric acid. The first 10 ml. of eluate are collected in a graduate, at which time the flow rate is determined. After this, 1-ml. fractions are collected in vials, dried, and titrated with carbonate-free 0.02*N* sodium hydroxide. Phenolphthalein is used for titrating the phosphoric acid peak, phenolsulfonephthalein for the other peaks. A modification of the Technicon fraction collector for collecting two hundred fractions in 25 (O.D.) × 50 mm. shell vials, warm-air drying chambers, vial racks, and titration apparatus have been described elsewhere (*21*). Adjusting the fraction volume may be facilitated by marking the first few vials at the 1-ml. level. Large parallax errors are avoided by the use of a level board holding a slot at one end and a dark background at the other end against which to view the meniscus.

Results. The batch of resin used for developing the analytical procedure and for the acid-recovery studies described below dated from February 1957. To determine the effects of age and batch of resin upon the analytical results, a new batch (purchased in October 1960) was tried in one run. Although the new batch (also nominally −400 mesh) had a somewhat smaller particle size as used, when tried with the same potato extract it gave an elution titration curve almost identical to that given by the old resin, both as to the size and the position of each acid peak. A typical titration curve is shown in Figure 1.

Recovery of the acids from the resin column was determined by preparing solutions of authentic acids, taking two equal aliquots of each acid, titrating one set of aliquots separately, and running the other as a mixture on the column. The quantities of acids used approximated those found in potato extracts. Table II gives the recoveries obtained in several such analyses and the standard errors. The table shows that the method is most precise for malic and citric acids. The method is still fairly precise for glutamic, aspartic, phosphoric, and oxalic acids, but not for pyroglutamic acid.

To determine the efficiency of the extraction procedure a potato sample was divided into two parts and each part extracted. Each successive batch of solvent used in extracting one part was analyzed. The results (Table III) show that most of the acids are obtained completely by two cold extractions, but that citric, phosphoric, and the unidentified acid require longer, or high temperature extractions. This is similar to the findings of Talley *et al.* (*23*) with amino acids in potatoes, and indicates that incomplete extraction will give not only smaller amounts of acids but also a false picture of their abundance relative to each other.

The batches of solvent used in extracting the second half of the sample were combined in the usual manner and then analyzed. The last two columns in Table III show that the total acid values are similar whether the batches are analyzed separately and the results totaled or whether the total extract is analyzed. This indicates that the recovery figures are valid for the acid quantities tested and also for smaller quantities.

A quantitative study of the major organic acids of the potato as related to growth and storage conditions is in progress and will be reported in a later publication.

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Literature Cited

(1) Andreotti, R., *Ind. conserve* (*Parma*) **31,** 111 (1956); **32,** 83 (1957).
(2) Aronoff, S., Vernon, L., *Arch. Biochem.* **28,** 424 (1950).
(3) Bate-Smith, E. C., Hughes, J. C., Swain, T., *Chem. & Ind.* (*London*) **1958,** 627.
(4) Bennett, G. M., Yuill, J. L., *J. Chem. Soc.* **1935,** 130.
(5) Bulen, W. A., Varner, J. E., Burrell, R. C., *Anal. Chem.* **24,** 187 (1952).
(6) Burton, W. G., "The Potato," p. 159, Chapman & Hall, Ltd., London, 1948.
(7) Busch, H., Hurlbert, R. B., Potter, V. R., *J. Biol. Chem.* **196,** 717 (1952).
(8) Craft, C. C., Siegelman, H. W., Butler, W. L., *Am. Potato J.* **35,** 651 (1958).
(9) Curl, A. L., Nelson, E. K., *Ibid.*, **17,** 328 (1940).
(10) Emilsson, B., *Acta Agr. Scand.* **3,** 328 (1953).

Table II. Recovery of Known Acid Mixtures from Ion Exchange Columns

Acid	No. of Replicates	Av. Recov., %	Std. Error
Glutamic	8	104	0.9
Aspartic	7	92.6	0.9
Pyroglutamic	7	112	4.3
Malic	8	96.8	0.4
Citric	8	98.3	0.3
Phosphoric	8	99.7	1.0
Oxalic	8	102	1.2

Table III. Acids Extracted by Successive Batches of Solvent

(Per cent of total acids present)

Acid	Cold Extractions 1	2	3	4	5	Hot Extractions 1	2	Total Acids[a] I[b]	II[c]
Glutamic	97	3						0.064	0.066
Aspartic	89	11						0.054	0.053
Pyroglutamic	95	5						0.043	0.050
Malic	100							0.042	0.045
Citric	54	32	12	1.8	0.9			0.338	0.343
Phosphoric	85	8.8	2.9	Trace		1.5	1.5	0.068	0.072
Oxalic	85	15						0.020	0.022
Unidentified	46	26				8.9	19	2.02[d]	1.87[d]

[a] Per cent on fresh weight of peeled potatoes.
[b] By adding analyses of the individual solvent batches.
[c] By analyzing the total extract.
[d] Milliequivalents per cent.